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MEETING REPORT

Models for Evaluating Agents Intended for the Prophylaxis, Mitigation and Treatment of Radiation Injuries Report of an NCI Workshop, December 3–4, 2003

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To develop approaches to prophylaxis/protection, mitigation and treatment of radiation injuries, appropriate models are needed that integrate the complex events that occur in the radiation-exposed organism. While the spectrum of agents in clinical use or preclinical development is limited, new research findings promise improvements in survival after whole-body irradiation and reductions in the risk of adverse effects of radiotherapy. Approaches include agents that act on the initial radiochemical events, agents that prevent or reduce progression of radiation damage, and agents that facilitate recovery from radiation injuries. While the mechanisms of action for most of the agents with known efficacy are yet to be fully determined, many seem to be operating at the tissue, organ or whole animal level as well as the cellular level. Thus research on prophylaxis/protection, mitigation and treatment of radiation injuries will require studies in whole animal models. Discovery, development and delivery of effective radiation modulators will also require collaboration among researchers in diverse fields such as radiation biology, inflammation, physiology, toxicology, immunology, tissue injury, drug development and radiation oncology. Additional investment in training more scientists in radiation biology and in the research portfolio addressing radiological and nuclear terrorism would benefit the general population in case of a radiological terrorism event or a large-scale accidental event as well as benefit patients treated with radiation.

INTRODUCTION

The expanding role of radiation therapy in cancer treatment along with the threat of nuclear or radiological terrorism creates new imperatives for discovering and developing agents for prophylaxis, mitigation and treatment of radiation injury. The choice of model systems and procedures is crucial to the success of these efforts (Fig. 1). A workshop, ''Models and Procedures for Evaluating Radioprotectors,'' sponsored by the Radiation Research Program of the National Cancer Institute (NIH, DHHS), was held on December 3–4, 2003, to recommend procedures for evaluating agents, selecting appropriate model systems, and validating the model systems. A brief report of the workshop has been published (*1*).

The mechanisms through which radiation injury becomes

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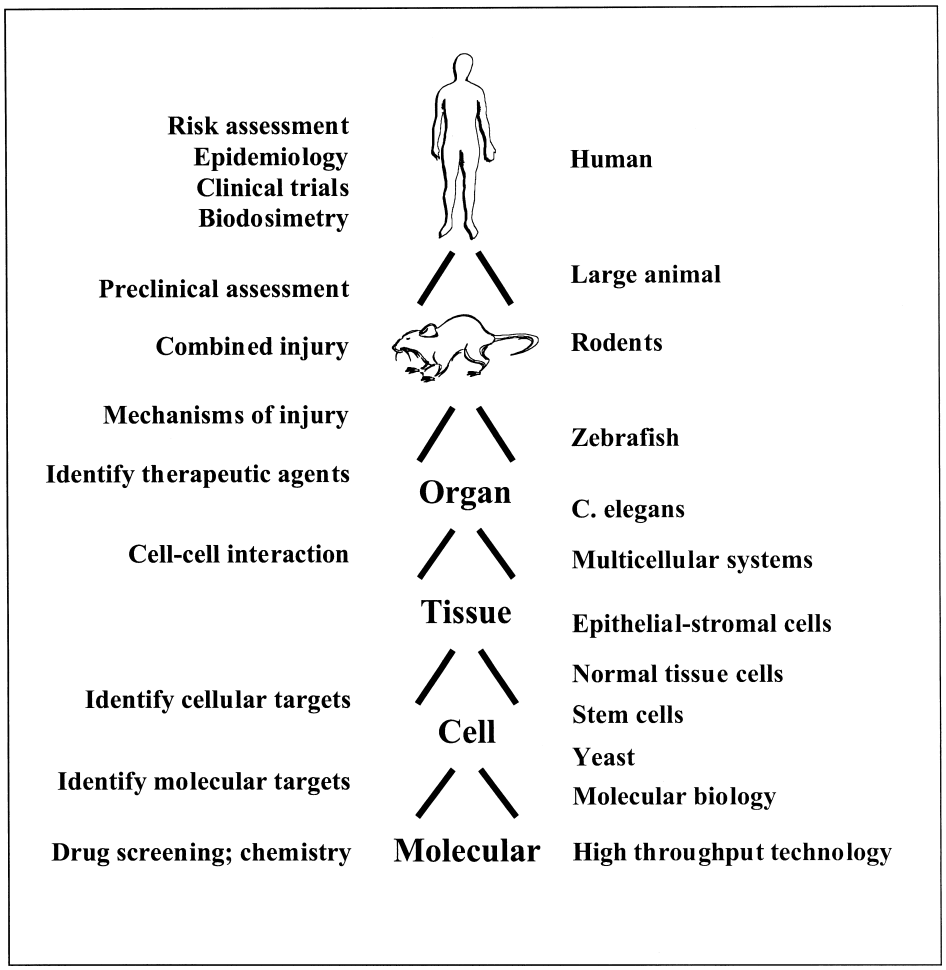

FIG. 1. The hierarchy of model systems for developing approaches to the prophylaxis, mitigation and treatment of radiation injuries.

manifest are not fully understood. They vary from tissue to tissue and depend on the circumstances of the exposure, such as dose of radiation, protraction of exposure, and concomitant exposure to other noxious agents or tissue trauma. Approaches to the prophylaxis, mitigation and treatment of radiation injury are many and varied. As a result, it is very unlikely that any single model system will be adequate for assessing all potential classes of agents.

The workshop focused on five organ systems that were considered to be of highest priority: hematopoietic, gastrointestinal, central nervous system, kidney and lung. At the workshop, it became clear that skin and soft tissue injuries could also occur in radiation accidents or terrorist events; although the topic was not discussed in detail, it has been included in this report. It is hoped that therapeutic approaches and principles developed in these systems could be extended to other organ systems.

The workshop did not address physical barriers (shielding), stem cell transplants, chelators that facilitate excretion of radioisotopes from the body, or agents (e.g. potassium iodide) that block uptake of radioisotopes by tissues. These have important applications in preventing or reducing the consequences of radiation exposure, and they can be considered among the countermeasures to radiation exposure; however, they were not the focus of the drug development effort proposed in this workshop.

Prophylaxis/Protection, Mitigation and Treatment

The terms ''protector'' and ''radioprotector'' have been used for many decades by the radiobiology community, primarily to refer to free radical scavengers that prevent the fixation of the initial radiochemical events after radiation exposure. It is now clear that potentially useful agents may act through a variety of other mechanisms (*2*). The workshop participants therefore recommended using terminology that is congruent with that used in medicine in reference to infectious agents, according to the time an intervention is to be administered: Prophylactic agents/protectors are given before radiation exposure; mitigators are given during or shortly after exposure, before the appearance of overt evidence of injury; and treatments are given after overt symptoms develop (Fig. 2). All of these classes are considered countermeasures for nuclear/radiological terrorism or

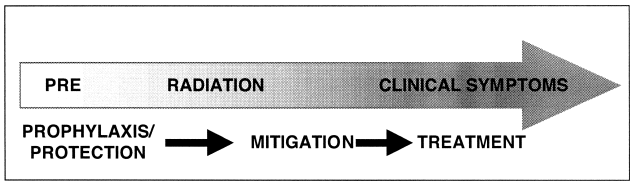

FIG. 2. Recommended terminology for therapeutic approaches to normal tissue radiation injuries.

for radiation accidents, where tumor protection is not an issue; some may also be applicable to radiation oncology. Conversely, an agent designed for the prevention or mitigation of injury in cancer patients treated with high total doses of fractionated radiation may not necessarily be appropriate for use in whole-body exposures to moderate doses of radiation, where injury involves multiple systems. In addition, the medical management of patients exposed to ''dirty bombs'' or nuclear devices may be complicated by traumatic and thermal injury.

All three approaches have been assessed in clinical or preclinical studies. Prophylactic agents include free radical scavengers, such as amifostine (*3*), that act on the initial radiochemical events and thus must be present at the time of irradiation. Mitigators, where treatment can be started after irradiation, include angiotensin-converting enzyme inhibitors (ACEI) in mitigation of radiation-induced lung (*4*), renal (*5*) and neurological (*6*) injuries. Treatments include pentoxifylline for treatment of radiation fibrosis (*7, 8*) and the use of hematopoietic growth factors that facilitate recovery from radiation-induced hematological injury (*9*).

HEMATOPOIETIC TISSUES

Radiation Response of the Hematopoietic System

Radiation injury to the hematopoietic system occurs in whole-body exposures, in partial-body exposures involving a substantial proportion of the bone marrow, when radiation is given prior to bone marrow transplantation (BMT), and in combined-modality therapy for cancer. Symptoms occur at doses of >1–2 Gy, and the 50% lethal dose ($LD_{50/60}$) after an acute exposure in humans is approximately 3.5–4.5 Gy. Deaths occur within 60 days (*10, 11*). Cytopenias develop as a result of death and normal attrition of mature, functional blood cells and failure of replacement because of depletion of hematopoietic stem cells and precursor cells. Lymphocytes decline within hours of exposure, platelets and granulocytes within days, and erythrocytes within weeks. Both the temporal pattern and extent of cytopenia roughly correlate with exposure level and prognosis (*12–14*).

Models for Studying Therapies for Radiation Injuries to the Hematopoietic System

Assays in mice based on survival, marrow repopulation *in vivo*, and *in vivo*/*in vitro* colony formation have provided the primary tools for the identification and analysis of clonogenically active subpopulations (*15–19*). Transplantation protocols demonstrate the regenerative power of marrow subpopulations, whereas colony-based assays establish the various progenitor subtypes, clonogenic potentials, and patterns/processes of lineage commitment and differentiation. Because of the importance of the stroma in supporting proliferation of hematopoietic stem cells (*20*), *in situ* assessment of agents will be essential to evaluating the clinical potential of agents.

Because of the hierarchical nature of the hematopoietic system, the multiple cell types involved, and the rarity of the stem cells, molecular and biochemical studies require rigorous cell isolation procedures to obtain homogeneous cell populations. cDNA differential expression array technologies can be used to identify the stem cell and progenitor marrow subpopulations in humans and animals (*21*). So far, little progress has been made in applying these techniques under the disequilibria after ionizing radiation.

The hematopoietic system can also be studied clinically and experimentally after either lethal or sublethal radiation exposures using molecular, cell/tissue-based and organ/system assays. These include procedures for cell identification, quantification and/or functional analyses. For general hematopoietic system status evaluations, standard blood hemograms, in particular CBCs and differentials, are routinely performed by electronic counting methods and will be useful for triage.

Current Status of Therapies for Radiation Injuries to the Hematopoietic System

BMT has been tried in some cases, such as the Chernobyl reactor accident. However, it has not been particularly successful, and it would be difficult to apply in emergencies when large numbers of people are exposed to radiation (*22, 23*). Therefore, other radiation countermeasures are needed.

Amifostine (WR-2721), the only prophylactic agent that has been approved by the Food and Drug Administration (FDA) for human use, is currently indicated only for reducing the incidence and severity of xerostomia in head and neck cancer patients treated with radiotherapy (*3, 24*). It has not yet been approved as a prophylactic for radiation injury of the hematopoietic system. Although a high dose of amifostine is an effective systemic protectant of the hematopoietic system when given prior to acute irradiation, the associated toxicity limits its use (*25, 26*). Subcutaneous routes of administration or rapid intravenous infusion may have adequate radioprotective efficacy without some of the toxicities associated with slow intravenous administration (*3, 27*).

Several recombinant growth factors and cytokines (G-CSF, pegylated G-CSF, GM-CSF, IL11) have FDA approval for chemotherapy-induced or etiologically undefined myelosuppression, but not for radiation-induced myelosuppression, even though they have proven therapeutic value for

injuries to the hematopoietic system after irradiation in mice (*9, 28, 29*). This drug-labeling issue is currently being resolved by the Center for Disease Control (CDC) in cooperation with the FDA and other interested federal agencies (*30*).

Stem cell factor (SCF) can block radiation-induced apoptotic signals through the FAS (CD95) pathway in subpopulations of hematopoietic stem cells (*31*). In mice given SCF 20 h before whole-body irradiation, the $LD_{50/30}$ was 25% greater than that in placebo-treated controls (*32*). Some of the survival benefit of SCF may have occurred from its protective effect on vital organ systems other than the hematopoietic system.

Other new classes of therapeutic agents showing promise include: androstene steroids, such as 5-androstenediol (AED) (*33*), fibroblast growth factors such as KGF (*34*), IL11 (*28*), and angiotensin peptides (*35*).

GASTROINTESTINAL (GI) SYSTEM

Radiation Response of the Gastrointestinal System

The intestine is a critical normal structure for patients undergoing radiation therapy for abdominal and pelvic malignancies (*36, 37*). The intestine could also be a critical organ in persons exposed in radiological terrorism and radiation accidents, as improved supportive therapies for hematopoietic system injury have made bone marrow toxicity more manageable. Because of the involvement of the intestine in bacterial translocation, sepsis and multiple organ dysfunction syndromes, the intestine may be particularly important in situations in which radiation exposure is combined with other forms of injury, a very likely scenario in nuclear warfare, radiological terrorism with an improvised nuclear device, or radiation accidents.

Acute GI injury occurs after whole-body doses of 3–15 Gy and, depending on dose, is characterized by nausea and vomiting, loss of appetite, diarrhea, luminal hemorrhage, loss of ionic and electrolyte balance, dehydration, infection, emaciation and death (*38, 39*).

In rodents, doses at the upper end of this range usually result in death within about 1 week after irradiation due to severe damage to the mucosal lining of the GI tract (*39*). Intensive supportive care with antibiotics, fluid and electrolyte replacement, etc. can prevent early death from this syndrome in human victims of radiation accidents, but these patients may die later from damage to other organs (*38, 39*).

Models for Studying Therapies for Radiation Injuries to the GI System

GI radiation responses have been studied in animal models ranging from fish to nonhuman primates. Mouse models provide greater opportunity for mechanistic studies due to the availability of genetically modified animals. On the other hand, results generated in rats may be more clinically relevant, since the radiation-induced fibrotic and immunological alterations in rats more closely resemble those observed in humans. The exception to the preference for rodents may be studies of the effects of radiation on GI motility (e.g. the prodromal syndrome), which have largely been conducted in dog models (*40, 41*). An extensive discussion of considerations for selecting among the many different animal models and different exposure models has been published elsewhere (*42*).

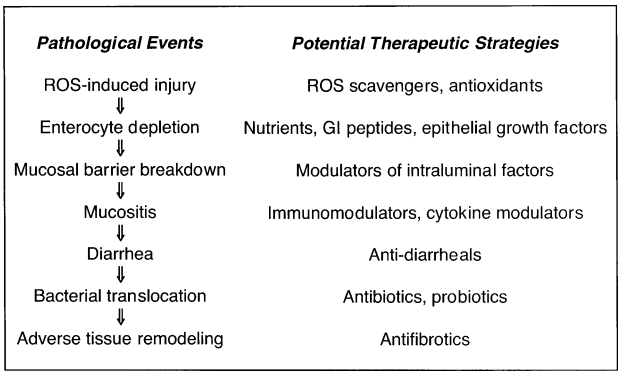

FIG. 3. The sequence of pathophysiological events involved in intestinal radiation injury and potential protective strategies that may be applied at each step.

Current Status of Therapies for Radiation Injuries to the GI System

Intestinal radiation toxicity develops as a chain of events, each stage of which is potentially amenable to modulation, as shown in Fig. 3. Many of the interventions listed in the figure are effective in animal models (*43*). According to a recently published evidence-based review of the clinical literature, the only interventions that have been shown to ameliorate acute intestinal injury in controlled or quasi-controlled studies are sulfasalazine, octreotide, amifostine and, for radiation proctitis, misoprostol enemas (*44*). In the treatment of chronic radiation proctitis, there is evidence to support the use of sucralfate enemas, short-chain fatty acid enemas, and coagulation therapy (*44*). Interventions that have undergone randomized testing and have been shown to be ineffective in acute intestinal radiation injury include oral glutamine, oral sucralfate and certain sulfasalazine derivatives (*44*). A clinically oriented overview of current and evolving management strategies in radiation injury of the intestine has recently been published (*45*).

Endothelial cells in the vasculature supporting the crypts and villi of the small intestine of mice are highly prone to radiation-induced apoptosis after a large single dose of radiation, but these cells can be protected by treatment of the animals with FGF2 (*46*). Moreover, FGF2 protected the animals against radiation-induced GI injury, suggesting that at such high radiation doses, dysfunction of the vasculature can reduce the ability of the crypts to regenerate from a few surviving cells (*47*). In chronic radiation toxicity, microvascular injury may also be key to the unique self-per-

petuating nature of radiation fibrosis (*48, 49*). Interventions aimed at restoring the thrombohemorrhagic balance are emerging as promising strategies to ameliorate both acute and chronic intestinal radiation toxicity (*50*).

In animal studies, a variety of cytokines have been reported to provide protection against both lethality and crypt cell depletion, including IL1A and B, SCF, IL11, TGFB3 and KGF (FGF7) (*39, 47, 51*). Conversely, IL12 has been reported to sensitize mice to GI injury (*52*). The timing of administration of these cytokines is important for the effect observed. Although in most cases the cytokines were given before irradiation, in one study FGF given after irradiation protected oral mucosa of mice (*51*). Other agents that have been reported to provide protection from GI effects of radiation in animals include amifostine (*53–56*), antioxidants (*57–59*), elemental diets (*60*), and MnSOD administered in an HSV viral carrier (*61*). Inhibitors of TP53 or the absence of TP53 has been shown to protect mice against hematopoietic injury (*62*) but does not protect the GI system from higher radiation doses (*63*).

Some clinical and animal studies have shown a modest protective effect of conventional anticoagulants, but the results to date have been mostly unimpressive and inconsistent. This may be a result of the use of non-specific drugs with multiple actions and use of drugs with dose-limiting side effects (bleeding), and, importantly, too narrow a focus on restoring the thrombohemorrhagic balance without considering the cellular effects of thrombin and the anti-inflammatory properties of the thrombomodulin-protein C system. Strategies that are more likely to be effective are those that directly target the thrombomodulin-protein C pathway, such as interventions aimed at increasing the endogenous expression of endothelial thrombomodulin, administration of exogenous soluble thrombomodulin, administration of recombinant activated protein C, or pharmacological activation of endogenous protein C [reviewed in ref. (*50*)]. Moreover, unlike the administration of proangiogenic factors, strategies that restore thrombohemorrhagic homeostasis would likely exert antitumor effects and thus not raise concerns of lack of differential protection when used in cancer treatment (*64–72*).

Since some of the effects of radiation on the GI tract are due to altered neuroendocrine control of contractile activity and neuroimmune interactions, it is possible that neurotransmitters or neurotransmitter inhibitors may also have a role (*41*).

CENTRAL NERVOUS SYSTEM (CNS)

Radiation Response of the CNS

High doses of radiation (>15 Gy) to the whole head or whole body produce cerebrovascular damage resulting in death within 2 days. Radiation-induced CNS injury from lower doses or the highly fractionated doses used in radiotherapy can be manifest as intellectual impairment or neurological deficits (paralysis and sensory loss) that develop over a period of months to years. Since both forms of injury severely compromise the well being of affected individuals, they constitute one of the most dreaded complications associated with cancer therapy for brain tumors and CNS prophylaxis for leukemia and high-risk solid tumors. The need to both understand and minimize the side effects of brain irradiation is urgently needed because of the increasing number of patients with secondary brain metastases that require treatment with large-field or whole-brain irradiation. Brain metastases occur in 20 to 40% of cancer patients (*73*), making this the second most common site of metastatic cancer, the most common neurological manifestation of cancer, and a cancer problem more common in incidence than newly diagnosed lung, breast or prostate cancer. Currently, approximately 170,000 patients with cancer per year receive large-field or whole-brain irradiation for management of brain metastases.

While high doses of radiation can result in significant morphological and functional alterations, exposure of the brain to lower doses can lead to cognitive impairments without inducing significant tissue destruction (*74, 75*). This type of CNS injury is manifest in long-term cancer survivors and may be significant after a radiation accident or a terrorist incident. Cognitive impairments often involve deficits in learning, memory and spatial information processing, functions associated with the hippocampus (*74–76*). Functional properties of neurons in the hippocampus are rapidly altered by low to moderate doses of radiation (5–10 Gy). These changes are dependent on dose, dose rate and time after exposure. Changes in the neuronal microenvironment are likely to underlie these effects (*77*). The hippocampus is also a site of active neurogenesis (*78, 79*), with new neurons produced in the hippocampal dentate gyrus throughout life (*78, 79*). The stem/precursor cells responsible are extremely sensitive to radiation, showing a steep apoptotic response after relatively low radiation doses ($\leq$2 Gy) (*80, 81*). These acute changes in the precursor population persist, leading to a dose-dependent decrease in the production of new neurons (*81*), which may be mediated in part through an inflammatory/redox-regulated process (*81, 82*). Recent data suggest that altered neurogenesis may play a contributory role in the cognitive impairments seen after exposure to ionizing radiation (*81, 83, 84*).

Models for Studying Therapies for Radiation Injuries to the CNS

Data have been collected from Japanese atomic bomb survivors, but most of the information available regarding radiation effects in the CNS have come from clinical and experimental studies that primarily involve relatively high doses. However, some *in vivo* studies have explored more moderate doses of radiation (*77, 80, 81*). The relevance of *in vitro* models is limited because of the complexity of the neuronal system as reflected in the relative insensitivity to

radiation *in vitro* (*85, 86*). Experimental studies, mostly conducted in rodent models, and retrospective clinical reviews have largely focused on changes in vascular and glial components, and considerable data exist on the effects of total dose, dose rate or fractionation and time after irradiation (*87–91*). In general, after high doses of radiation, neurological deficits develop after a dose-related latent period and become progressively more widespread with time.

Current Status of Therapies for Radiation Injuries to the CNS

While corticosteroids are frequently used for symptomatic treatment of radiation injury, few studies have systematically addressed their potential for treatment of overt CNS injury (*92, 93*). However, progress in neurobiology has opened new research directions. Recent studies generated exciting preliminary data in rodent models addressing the role of progenitor cell transplantation and neurotropic growth factors, such as platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF), in ameliorating CNS injury (*94, 95*). If confirmed in large animal models, these findings could change the standard of care of cancers located in the vicinity of the CNS.

Presently there are no established means to ameliorate or treat the cognitive impairments observed after radiation exposure. Given the potential role of proinflammatory cytokines in radiation brain injury (*96, 97*), the impact of specific cytokines on neurogenesis (*98*), and the apparent role of inflammation in conjunction with radiation-induced changes in neurogenesis, it is possible that anti-inflammatory approaches may be useful (*81, 82*). Additionally, the radiation response of neural precursor cells *in vitro* and *in vivo* is associated with an elevated and persistent oxidative stress (*99*), suggesting that antioxidant treatment may modulate the effects of radiation on precursor cells and, ultimately, the development of cognitive impairment.

LUNG

In 2003, more than 400,000 patients were diagnosed with thoracic malignancies in the U.S. The majority of these patients will receive radiation therapy as part of the treatment regimen for their cancer. For patients treated with intrathoracic malignancies, the lung receives a range of doses, from the low doses one might encounter in radiological or nuclear terrorism to high tumoricidal doses. For this reason, clinical cancer treatment may provide a setting in which to evaluate mitigation and treatment of radiation-induced lung injury.

Radiation Response of the Lung

The lung is very sensitive to radiation-induced injury (*100*). Up to 20% of patients receiving radiation to the chest will develop symptomatic injury, and an even greater percentage will experience asymptomatic reduction in pulmonary function (*101*). Injury occurring within 3–6 months after exposure typically manifests as pneumonitis, with shortness of breath, cough and occasionally fever (*101*). Histologically, it is characterized by interstitial and airspace edema, inflammatory infiltrate (mostly macrophages), and loss of epithelial cells (*100, 102*). Injury occurring at later times usually manifests as progressive shortness of breath with diffuse interstitial fibrosis, focal scarring and loss of alveoli (*100, 101*). In both animal models and humans, the development of injury depends on the total dose of radiation, the dose per fraction, and the time over which the radiation is delivered (*101*). For humans, a single dose of 9.3 Gy to the whole lung would be expected to be fatal 50% of the time (*100*). In contrast, 24.5 Gy in 1.8–2-Gy fractions to the whole lungs would have a 50% chance of resulting in symptomatic injury, which usually would not prove fatal (*103*).

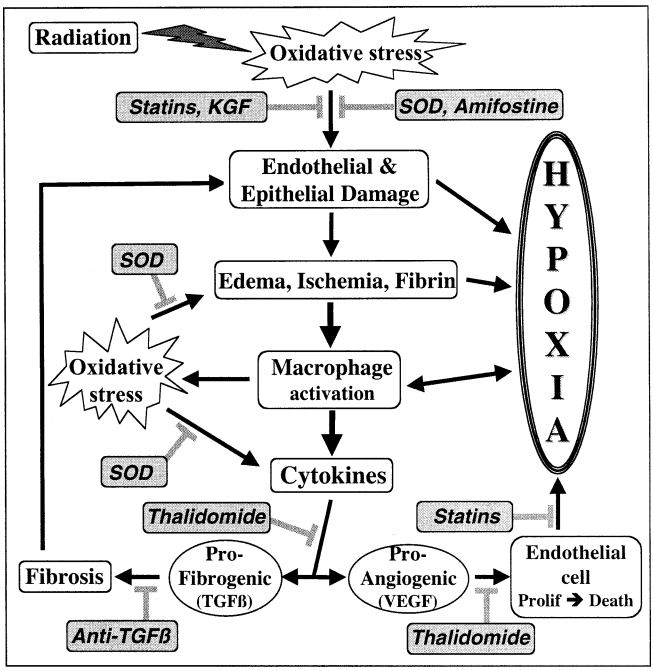

FIG. 4. Pathogenesis of radiation pneumonitis showing the possible steps where protection, mitigation or treatment approaches could operate.

Although clinical symptoms and/or radiographic findings may not develop for weeks to months after exposure, the molecular events underlying radiation injury begin to occur immediately (*104*). Radiation-induced pulmonary fibrosis and alveolitis are multifaceted pathological processes with different points of progression leading to loss of function, morbidity and death. Radiation triggers a cascade of molecular and cellular events that proceed during a clinically latent period (Fig. 4). This is an active process involving a variety of cell types (e.g. endothelial cells, macrophages, epithelial cells and fibroblasts), proinflammatory and profibrotic cytokines (e.g. IL1, TNFA and TGFB), and the stimulation of gene products and transcription factors (e.g. EGR1, NFKB, JUN and FOS). These processes persist well

beyond the end of exposure to radiation, and may create a chronically hypoxic environment (Fig. 4) which may be important in perpetuating the aberrant wound-healing response characteristic of this late injury (*101*). Recently, several radiation-specific gene loci have been identified which appear to be implicated in susceptibility to radiation-induced fibrosis (*105*).

Models for Studying Therapies for Radiation Injuries to the Lung

In general, most animals display a higher tolerance to pulmonary radiation than do humans. The most commonly used models have been rodents (rats and mice), although larger animals, such as pigs, may be more similar structurally to humans. End points used in rodent studies include breathing rate and tidal volume, bronchoalveolar lavages, histological assays and survival (*106, 107*).

Current Status of Therapies for Radiation Injuries to the Lung

Current therapies to limit alveolitis have had limited success in the clinic, although corticosteroids have been used successfully in many cases (*108, 109*). There are no effective therapies to treat lung fibrosis. The free radical scavenger amifostine has been evaluated clinically with mixed results (*110*). ACEI and angiotensin II (AII) receptor antagonists have been used successfully in animals (*4, 111*). Although a retrospective clinical study of incidental ACEI use in patients receiving thoracic irradiation found no benefit (*112*), a randomized, placebo-controlled trial of ACEI for the mitigation of radiation-induced lung injury was launched by the Radiotherapy Oncology Group in early 2004.[2] Other promising strategies currently in preclinical evaluation include anticytokines (*113*), mucosal protectants [e.g. KGF (*114, 115*)], and redox modulators [e.g. superoxide dismutase and its mimetics (*116, 117*)].

KIDNEY

Radiation Response of the Kidney

The kidneys are among the most radiosensitive organs. When both kidneys are irradiated, the tolerance dose for daily fractionated radiotherapy is 20–25 Gy (*118–120*). Radiation nephropathy may occur after radiotherapy for Wilm's tumor, neuroblastoma, lymphoma, and testicular and ovarian carcinoma. Over the last decade, radiation nephropathy has emerged as a major complication of BMT when total-body irradiation (TBI) is used as part of the conditioning regimen, where it is reported to occur for fractionated doses as low as 10–12 Gy (*119, 121, 122*). More recently, radiation nephropathy has also appeared as a complication of radionuclide therapy (*123, 124*), where it is reported to occur at doses as low as 7.1 Gy (*125*). In humans and animals, radiation nephropathy is characterized by early proteinuria followed by slowly progressing azotemia and hypertension that lead eventually to renal failure (*5, 126, 127*). Histopathologically, there is glomerular and tubular injury and subsequent progressive scarring (*126*).

Models for Studying Therapies for Renal Radiation Injuries

Radiation nephropathy has been studied in mice (*128–130*), rats (*127, 131*), pigs (*132*), dogs (*133, 134*) and nonhuman primates (*135*). With the possible exception of the mouse, which is notably resistant to renal irradiation (*136, 137*), all the species tested show physiological and histopathological changes that resemble those seen in human radiation nephropathy. In general, the animal species of choice would be the smallest that is suitable for the end point to be studied.

End points for assessing the efficacy of therapies range from physiology (*127*) to histopathology (*137*). The earliest physiological change that is clearly linked to renal fibrosis and end-stage renal disease is the development of azotemia (*138, 139*). Investigators have assessed some early (within 10 days) radiation responses in rodent kidney, including changes in gene expression (*140*), changes in glomerular permeability (*136*), and development of markers of DNA oxidation (*141*), but their usefulness as predictors of the severity of late renal injury has not been established. Renal cells [e.g. mesangial and proximal tubule epithelial cells (*142, 143*)] can be grown and irradiated in cell culture, but as yet the responses of cultured cells cannot be used reliably to assess therapies, because the mechanisms of action of therapies probably depend on interactions at the tissue or organism level (*144*).

Current Status of Therapies for Radiation Injuries to the Kidney

A number of approaches to the prophylaxis, mitigation and treatment radiation nephropathy have been developed (Fig. 5). There is preclinical evidence that established radiation nephropathy can be treated with ACEI or AII receptor antagonists (*5*), and the clinical efficacy of these agents for treatment of radiation nephropathy has now been established (*145, 146*). There is also experimental evidence that ACEIs (*5*), AII receptor antagonists (*5*), and dexamethasone (*131*) can be used to mitigate the development of radiation nephropathy. The clinical efficacy of these agents for mitigation has not yet been proven, but a prospective clinical trial of the use of an ACEI to mitigate radiation nephropathy in BMT patients is in progress (*5*). In addition, there is preclinical evidence that chronic oxidative stress plays a role in radiation-induced renal injury, suggesting that antioxidant therapy (e.g. SOD) might be useful for mitigation or treatment of radiation injuries

[2] RTOG-0123: A Phase II randomized trial with captopril in patients who have received radiation therapy ± chemotherapy for stage II–IIIB non-small cell lung cancer, stage I central non-small cell lung cancer or limited-stage small cell lung cancer

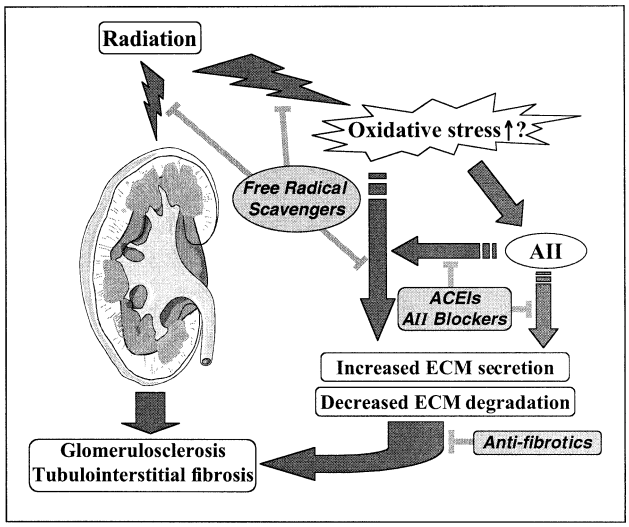

FIG. 5. Pathogenesis of radiation nephropathy showing the possible steps where protection, mitigation or treatment approaches could operate.

(*141*). In other preclinical studies, both cysteamine (*147*) and amifostine (*148, 149*) have been shown to be effective renal radioprotectors.

SKIN AND SOFT TISSUES

Radiation Response of the Skin and Soft Tissue

Skin is susceptible to damage from radioactive isotopes carried by fallout from nuclear weapons or radiological dispersion devices (''dirty bombs''). A high incidence of radiation-induced cutaneous abnormalities was found in the 15-year follow-up of individuals heavily exposed during the Chernobyl incident (*150*), and severe skin injuries resulted from the nuclear criticality accident in Japan in 1999 (*151*).

The response of the skin to ionizing radiation involves several distinct phases, and the severity depends on dose and conditions of exposure. Acute erythema is seen within hours after doses of 2–6 Gy. This phase is transient and often subsides within 48 h. It is generally considered to be due to release of vasoactive agents, including radiation-induced proinflammatory cytokines, rather than to direct cell killing.

The main erythematous phase evolves 2–4 weeks after doses in the range of 5–10 Gy. This is precipitated by loss of functional skin cells and the failure of epidermal basal cells to replace them (*152*). Progressive epilation and suppression of sweat glands also occur during this phase. In humans, the main erythematous reaction has been reported to occur 30 days after 12.5 Gy (*153*). If stem cells in the basal layer of the skin recover in time, the lesion resolves. If too many are killed to allow timely recovery, dry or moist desquamation results, depending on the number lost. After 5 weeks, re-epithelialization is initiated and proceeds slowly from ''islands'' of clonogenic cells within the wound or from the margins. If the denuded site is in excess of 15 mm in diameter, repopulation becomes difficult (*152*), so the outcome is dependent on the area receiving high doses. If repopulation is compromised, secondary effects such as ulceration and infection are likely. Because the skin is a physical barrier, electrolyte and fluid loss and thermoregulation become issues. The skin also protects the body against infectious agents. The likelihood of infection is greatly increased if there is concomitant systemic immune suppression. Systemic immune deficits may also jeopardize the healing process in an irradiated site, since the immune and inflammatory cells play a major role in wound healing (*154*), although the inflammatory erythematous phase of the reaction may be lessened.

A late phase of erythema, edema, loss of pigmentation and dermal necrosis may occur starting 2 months after exposure doses around 15–20 Gy. This late phase is most likely due to loss of capillaries and decline in dermal blood flow (*155*), with probable development of hypoxic areas. Later still, atrophy and thinning of the dermal tissue can occur. In pig skin, two phases have been reported 3–5 months and 1 year after exposure to beta-particle emitters (*152*). Finally, telangiectasia is a very late effect involving dilation of the superficial dermal capillaries that is highly dependent on dose (*156*). The skin is, of course, particularly susceptible to trauma-induced precipitation of late radiation effects.

If the radiation energy is sufficiently high, damage to the underlying deep dermis may manifest itself as progressive fibrosis during the late delayed phase. Radiation-induced senescence of fibroblasts may play a role during this phase, with TGFB playing a role in causing cellular senescence and collagen deposition (*157*). The altered balance of cytokines, proteases and extracellular matrix (ECM) after irradiation affects collagen remodeling and the collagen subtypes that are produced (*158*) as well as the healing response (*159*). It is presumably this balance that dictates radiation-induced fibrosis.

Models for Studying Radiation Injury to the Skin and Soft Tissue

The thickness of the epidermal layers in humans varies threefold with body site, age and sex (*152*), which has implications for assessing the response to low-energy emitters. Pig skin is generally considered to be most similar to human skin in many features (*152, 160*), although there is variation among different types of pig. Much relevant information can be obtained using the mouse, of which genetically modified strains are available. For example, Smad3 knockout mice are protected against radiation-induced cutaneous injury, indicating involvement of the TGFB pathway (*161*). Halofuginone has been demonstrated to protect against tissue fibrosis through alteration of the Smad pathway (*162*). The mouse has also been used extensively for studies of soft tissue fibrosis with leg or skin contracture as an end point.

Recently, progress has been made with *in vitro* culture

systems, allowing the effects of radiation to be studied in human keratinocytes and dermal fibroblast cultures (*159*) either alone or together, for example in collagen sponges (*163*). Whole skin cultures have also been developed (*164*). The effects of radiation on vascularity, edema, cell proliferation, cytokines and collagen have been observed in such cultures over a period of weeks (*165*). Radiation-induced ICAM1 expression has been studied in the human split skin culture system (*166*). A number of studies have identified roles for cytokines in maintaining communication between cell types in the skin and in wound healing situations. For example, IL1 is a critical cytokine produced by keratinocytes that plays an important role in cutaneous responses, and TGFB has been shown to be elevated in early and late phases of cutaneous damage (*160*).

Current Status of Therapies for Radiation Injury to the Skin and Soft Tissue

One advantage of skin is that it is amenable to topical application of therapeutic agents. On the other hand, one of its disadvantages is that, because of its importance to host defense, wound healing is a highly redundant, complex process that makes it difficult to evaluate or specifically target involved molecules. Extensive desquamation is a major management issue requiring fluids, antibiotics and, where necessary, skin grafts. Topical application of evening primrose oil and other polyunsaturated fatty acids has been found to modulate cell proliferation in pig skin and to be beneficial in the case of radiation injury to the skin (*167*) and mucosa (*168*). Application of an emulsion containing trolamine affected the response of human skin cultures to radiation (*165*). COX2 inhibitors have been shown to reduce acute skin reactions and chemokine and receptor expression in mice (*169*). IL1 and TGFB can mitigate the effects of radiation on skin wound healing (*159*). Importantly, radiation fibrosis has been reversed in irradiated patients using liposomal Cu/ZnSOD (*170*) or pentoxifylline either alone (*8*) or in combination with tocopherol (*171*). Alteration in the balance of cytokines, proteases and extracellular matrix materials, which modify the phenotype of the fibroblasts within the irradiated site, are thought to mediate these effects.

MODEL SYSTEMS OTHER THAN WHOLE MAMMALS

Although countermeasures to radiation-induced normal tissue injury are thought to involve complex interactions at the tissue, organ and organism level, the workshop participants also discussed some model systems other than whole animals that might be used for mechanistic studies or drug development and screening (Fig. 1).

Yeast

The yeast *Saccharomyces cerevisiae* can be cultivated easily in large quantities and might be useful as a potential tool for high-throughput screening of therapeutic agents (particularly protectors/prophylactic agents). It has been used extensively to characterize biological processes, its genome has been sequenced, and precise gene disruptions can be performed with ease because it has a highly efficient mechanism of homologous recombination. Furthermore, many of the biological processes are conserved (e.g. double-strand break repair genes were originally characterized in yeast) (*172*).

The DEL assay for deletions in yeast chromosomes (*173*) could be applied as a screen to identify novel chemicals that can influence radiation sensitivity and/or acute (*174*) or persistent (*175*) genetic instability. A high-throughput assay platform (including the data evaluation software) is being developed (R. H. Schiestl, unpublished results), and such an approach might be used to discover new radioprotective agents.

Nematode

The nematode *Caenorhabditis elegans* provides an animal model with distinct advantages for the study of normal tissue function and pathophysiology (*176*). This microscopic worm grows from an embryo to an adult containing exactly 959 cells within 3 days. Because *C. elegans* is transparent at all stages of development, it has been possible to characterize in exquisite detail the division, migration, differentiation, fusion and death of every cell. The *C. elegans* genome was the first animal genome to be identified molecularly, and it contains orthologs of many mammalian genes in a tractable genetic system.

The applicability of the *C. elegans* model to the study of radiobiological processes is in its infancy. Nevertheless, much of the machinery for the sensing of DNA double-strand breaks, induction of cell cycle checkpoints, and induction of apoptosis appears to be evolutionarily conserved in the worm (*177*). Further, recent study has provided proof that *C. elegans* can be used to analyze complex radiobiological issues. Gartner *et al.* (*178*), and Kolesnick *et al.* (*179*) have described a radiation-activated pathway to apoptosis requiring cell cycle checkpoint genes and the genes comprising the conserved apoptotic machinery.

An example of how the worm could be used to evaluate effectiveness of new compounds or in the discovery phase is evinced by recent studies on the *C. elegans* homolog of c-Abl, ABL1 (*179*). c-Abl is a conserved non-receptor tyrosine kinase that integrates genotoxic stress responses, acting as a transducer of both pro- and anti-apoptosis effector pathways. The germline of worms homozygous for a deletion allele of *abl-1* displayed hypersensitivity to radiation-induced apoptosis. This phenotype could be mimicked in wild-type worms by treating them with the c-Abl inhibitor STI-571 (Gleevec) used in human cancer therapy. Two newly synthesized STI-571 variants and PD166326 had similar actions. While this example involved an antitumor therapy, similar approaches might be used to target con-

served genes and pathways involved in development of normal tissue damage after radiation exposure. Clearly, more work is needed to develop and validate this model for discovery of protectors/prophylactic agents, mitigators and treatments of radiation toxicity.

Zebrafish

The zebrafish, *Danio rerio*, has many of the advantages of simpler model organisms such as yeast and *C. elegans*, but unlike these organisms, it has a full complement of vertebrate organs, including a brain and spinal cord, chambered heart, digestive and excretory organs, and a hematopoietic system similar in many respects to that of mammals. Thus it is possible to investigate organ-specific effects of radiation that cannot be studied in lower eukaryotic models.

The zebrafish has a well-developed classical genetics, and extensive genomic resources are available (*180*). Homologs of proteins involved in repair of radiation damage in mammals have been identified in the zebrafish. Forward genetic screens in the fish are much faster and less costly than in the mouse. Rapid characterization of mutant phenotypes, particularly for traits expressed early in development, is aided by the physical accessibility and optical transparency of the embryo and early-stage larva and by the rapid developmental program, in which all major organ systems are formed and functional within a few days after fertilization. Artificial induction of parthenogenetic development permits identification of recessive alleles one generation earlier than is possible in a classical breeding screen. Together, these characteristics suggest that large-scale screening to identify new genes and pathways that influence organ-specific susceptibility to radiation injury should be feasible.

The U.S. Department of Energy has awarded funds under its Low-Dose Radiation Research Program for development of the zebrafish as a radiobiological model. Results reported at an October 2003 DOE contractors' meeting suggest that the developing nervous system of the embryo is particularly sensitive to radiation injury and may afford a model for the effect of radiation on neurogenesis in the mammalian brain (C. L. Bladen, W. S. Dynan and D. J. Kozlowski, unpublished results).

The hematopoietic system of the zebrafish has also been well characterized, and the biological effects of lethal doses of radiation and rescue by transplantation of hematopoietic stem cells have recently been reported (*181*). The use of adult zebrafish as a model to test the efficacy of SOD and small molecule mimetics for preventing radiation-induced fibrosis is also under active investigation (J. S. Greenberger, unpublished results).

Nonmammalian vertebrates such as the zebrafish can contribute to the discovery of new therapies by identification of specific proteins or signaling pathways as therapeutic targets, based on the results of genetic screening. Conventional high-throughput screening could then be used to identify drugs that interact with these targets. In addition, because zebrafish embryos and larvae can absorb or ingest compounds present in low concentrations in the water in which they develop (*182*), they could be used directly in high-throughput screens of chemical libraries for compounds that influence the development of radiation damage. Zebrafish strains are available that have been tagged with fluorescent transgenes in a number of cell lineages of interest to radiation biologists, including neuronal and hematopoietic precursors and vascular endothelial cells. These could be used for high-throughput *in vivo* screening to identify organ-specific radiation mitigators that modify the effect of radiation on proliferation of the fluorescent cell lineages.

Tissue-Specific Models in Cell Culture

Research using standard monolayer culture has generated a basic understanding of how cells can respond to radiation and has identified a variety of factors that influence the degree and type of response, including cell cycle distribution, the presence of cytokines and other factors, and interactions with other cells. However, by measuring individual biological events, one is unable to describe how the organism will respond to damage. Formerly there was an implicit assumption that the biological effects of radiation occur only in cells actually exposed. Over the last decade numerous studies have demonstrated that radiation effects extend beyond the irradiated cell and even the irradiated tissue (*183*). Furthermore, there is considerable evidence that a hierarchy of cell responses in tissues may ultimately direct tissue response to radiation (*184*), which would be unappreciated in single cell culture models. Indeed, the cell biology of irradiated tissues may be viewed as a coordinated multicellular damage response program in which individual cell contributions are directed toward repair of the tissue (*185*).

Nevertheless, it should be possible to develop culture assays aimed at answering specific questions. Physiologically relevant human cell culture models would provide better experimental flexibility than is available in animals and could provide a foundation for extrapolating from radiation responses in culture to those in humans. The behavior of individual cells is dictated by their interactions with each other, and this microenvironment is essential for functional organization and differentiation (*186*). To create a more accurate model, it may be necessary to embed cells in a reconstituted ECM, which could result in aspects of normal tissue behavior that are not apparent in conventional cell culture models. Studies using three-dimensional tissue culture models with reconstituted ECM have shown that tissue organization is necessary for cells to display appropriate tissue-specific differentiation and survival (*187*).

We must remain aware, however, that because the body integrates its many functions by a multitude of communi-

cation systems, cell culture systems cannot predict how a drug will affect an intact organism. Genetic similarities and differences among organisms revealed by genome maps have so far revealed little about how these are translated into the differences and similarities we can see among these organisms.

STRATEGIES

Screening of Potential New Therapies

High-throughput screening would be practical now for certain types of approaches, such as radioprotection of the hematopoietic system by free radicals (*3, 24*) or cytokines for protection of the GI system (*39, 47, 51*). High-throughput screening might also be practical in the near future for approaches such as the use of SOD mimetics (*116, 117*) or AII receptor blockers (*4*) for mitigation and treatment of radiation-induced lung injuries. Other potential targets common to several organ systems are TGFB (*157, 160–162*) and TP53 (*62, 188*). However, high-throughput screening of compounds is unlikely to lead to discovery of agents acting through previously unknown mechanisms. For example, screening systems designed to detect free radical radioprotectors would not have revealed that androstene steroids were effective against hematopoietic injury (*33*) or that AII receptor blockers were effective for mitigation radiation-induced lung injuries (*4*), since these agents are not effective in the types of assays and schedules used to detect free-radical radioprotectors. High-throughput assays in cell culture systems or in non-mammalian systems should be useful after organ-specific whole-animal models have identified specific targets and lead agents. However, agents that depend on physiological interactions at the organ or whole-animal level and agents that require metabolic activation could be missed in simpler systems.

Identification of New Therapeutic Targets

Basic research is needed to identify additional targets for design, synthesis and evaluation of small molecules for preventing damage or promoting healing. For example, chronic inflammation (*81, 82, 96, 97, 102, 104, 154*) and/or oxidative stress (*61, 99, 116, 117, 141, 170, 189*) appear to be involved in the development of late effects, so that proinflammatory cytokine signaling pathways and redox pathways are areas for developing interventions. This will require assays based on those specific pathways, including proof-of-principle assays *in vivo*. Agents that target tissue- or organ-specific mechanisms for development of radiation injury will require organ-specific procedures and models that have the relevant pathways.

Because understanding of the mechanisms of development of radiation injury in normal tissues is evolving, procedures, assays and model systems will evolve as well. However, the urgent need for agents to prevent, mitigate and treat radiation injury cannot wait for complete understanding of radiation injury and of models. Agents known to be effective in prophylaxis, mitigation and treatment of radiation injuries need to be tested in appropriate models to build a knowledge base for future testing of compounds.

The NCI Experience with Anticancer Agents

The Developmental Therapeutics Program (DTP) has long been the site of NCI drug discovery and preclinical development efforts. While not all the lessons learned from this experience are applicable to the discovery and development of radioprotectors, mitigators and therapeutics, there are a number of general principles that are worth considering. The DTP effort started as a mostly self-contained ''pipeline'' with compounds entering a primary screen and moving though confirmatory assays to animal models, to formulation, GMP synthesis [Good Manufacturing Practices, 21 CFR 210 (*190*)], pharmacology, toxicology and, ultimately, clinical trials. Work was done mostly in contract laboratories with project management by DTP staff. All the decisions regarding a compound's flow through this pipeline were made by DTP staff. There was, however, a perception in the research community that this process was rigid, limited and disconnected from the community's efforts. In response, the DTP effort has evolved a much more open and modular process. The same capabilities are still in place, but there is much greater flexibility in the choice of and access to services. There is still a primary screening service, but activity in this screen is no longer the primary criterion for later-stage development. Access to these development services can be obtained by peer review in the RAID program (Rapid Access to Intervention Development, an NCI program that provides resources for preclinical drug development; see http://resresources.nci.nih.gov/database.cfm?id=378), with the investigator deciding which services are appropriate for each particular project. Thus, for many projects, the DTP service may provide a crucial enabling role, but it is only one part of an overall development effort that is controlled not by DTP staff, but by the originating investigator. The data for hundreds of thousands of compounds have been made available to the research community, where possible, though the DTP web pages (http://dtp.nci.nih.gov/).

Pharmacology and Toxicity

New drugs often are dropped during development for animal toxicity (17%), human toxicity (16%), pharmacokinetics (7%) or lack of efficacy (46%) (*191*). Thus *in vitro* and *in vivo* models that are predictive of the human response are the most important factors that determine the clinical success of new drugs and must be carefully selected and their human predictivity validated, if possible. Kinetic and toxicity studies in normal animals are the final steps in the evaluation of new drugs before entry into the clinic. Experience has repeatedly shown that no one species may be predictive of all human toxicities and that not all human

toxicities may be seen in other animal species (*192*). Thus discovery and development must involve integrated studies of efficacy, pharmacology and toxicology.

Pharmacology, both pharmacokinetics and pharmacodynamics, must be used to determine the plasma or tissue drug levels (peak, area under the curve, threshold) required to have an impact on the target for efficacy, as well as for end points of toxicity. With newer molecularly targeted drugs, measurement of target modulation is also necessary. This requires the selection of which parameter to monitor as a measure of biological activity that is correlated with efficacy and the necessary development and validation of appropriate methods. Genomics and proteomics studies may assist in developing assays for use in preclinical and clinical studies. Then these data are used to design toxicology studies to determine whether effective concentrations can be attained safely using the optimal route and schedule. As in the efficacy studies, the toxicology studies also must include pharmacokinetics/pharmacodynamics, genomics and proteomics to determine whether there is a real therapeutic index, and whether efficacy and toxicity are induced by different mechanisms that might allow for separating toxicity from efficacy by changing schedule or mode of administration (*193, 194*).

NCI has found that the effects seen using the murine tumor or human tumor xenograft models do not correlate well with clinical anticancer activity (*195*). The human tumor xenograft model is relatively sensitive to the effects of many new cytotoxic agents, while the normal tissues of the murine host are relatively resistant to the toxic effects of such agents, creating an abnormally high therapeutic index (*196*). This may be less problematic in the evaluation of the efficacy and toxicity of protectors, mitigators and treatments for radiation effects where the efficacy involves tissue survival and function rather than tumor destruction. The one area in which the Toxicology and Pharmacology Branch of the DTP has been successful in using human tissue to predict human sensitivity relative to animal models is the use of an *in vitro* bone marrow assay developed under the Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) Programs (see http://grants2.nih.gov/grants/funding/sbir.htm). The recent development and validation of this *in vitro* assay using rodent, canine and human CFU-GM and other stem cells has demonstrated the utility of *in vitro* assays for correlating and predicting *in vivo* toxicities in animals and humans (*197*). The use of molecular end points to evaluate toxicity and for high-throughput toxicity screening has allowed the exploration of toxicity to be incorporated at an earlier stage in drug development. Thus the development of new *in vitro* assays to predict other dose-limiting toxicities such as cardiotoxicity, gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, neurotoxicity and pulmonary toxicity would be a very useful adjunct to the *in vivo* toxicology studies currently required. These assays would assist in the evaluation and prediction of human sensitivity and allow for more cost-efficient evaluations of numerous analogs prior to the selection of the ultimate drug development candidate. These assays could also lead to a more efficient use of animals in toxicity studies, thereby reducing the cost of drug development, while improving the predictability of these studies.

Regulatory Issues

Prior to conducting Phase I clinical trials of new therapeutic agents, an application for an IND (Investigational New Drug) must be submitted to the FDA (http://www.fda.gov/cder/handbook/dev_rev.htm). For 30 days after submission, FDA scientists review the application, with a focus on safety. At the end of 30 days, the IND is either found ''safe to proceed'' or placed on clinical hold. The latter situation is not typical or desirable for the developer or the FDA, but it is often necessary if insufficient safety data are provided. For this reason, adequate preclinical toxicology and pharmacokinetics are crucial components of the package, especially for NMEs (new molecular entities). The latter data are necessary to determine expected toxicities, a safe starting dose, routes of drug metabolism and elimination, and whether the therapeutically necessary drug level and duration of exposure can be achieved. Pilot batches of the agent must be synthesized in the amounts needed for a clinical trial, using GMP standards to ensure the required purity. Standards appropriate to the stage of product development can be obtained from the FDA Center for Drug Evaluation and Research (CDER).

The above may be less a barrier to the development of therapies for radiation injury than it appears. Some of the products currently under development for prophylaxis, mitigation or treatment of radiation injuries are already approved or licensed products, but they do not carry the precise radiation-related indication (e.g. some of the hematopoietic cytokines). In the latter case, substantial information about human safety and pharmacokinetics is already known, so the development program issue can be focused on demonstration of efficacy (*30*). Still other classes of approved products are currently in clinical use for the proposed radiation-induced injury, but again do not carry the precise indication. For example, AII blockers are approved for treatment of hypertension and are already in clinical use for treatment of radiation nephropathy (*145*), but they do not carry an explicit radiation-related indication. Such usage for individual patients would fall under the ''practice of medicine,'' which is not regulated by the FDA. However, there are strong public health reasons to continue development of these products such that formal approval of the radiation-related indication is achieved. These include use of the product during a mass casualty event, such as might occur as a consequence of terrorism.

Finally, although some drugs intended as medical countermeasures could not be ethically evaluated for efficacy in controlled clinical trials, they could be developed under the

Animal Efficacy Rule (*198*). However, the Animal Efficacy Rule has limitations. It applies only to establishment of efficacy of the therapy relative to the threat agent, and it does not apply to issues such as the clinical safety and pharmacokinetics of the countermeasure itself. In addition, the pathophysiology of the disease and the mechanism of action of the countermeasure must be reasonably well understood, which could be a challenge if the underlying process leading to the morbidity is not well understood, as with chronic radiation injuries. Animal efficacy studies intended to support the indication are to be conducted using Good Laboratory Practice (GLP) standards. GLP regulations [21 CFR 58 (*199*)] have been applied to nonclinical laboratories for over 25 years. There are resources available to assist the investigator in complying with these standards (http://www.fda.gov/ora/compliance_ref/bimo/glp/default.htm) and avoiding costly modifications that may not be necessary. The efficacy of the countermeasure must be established in more than one species (including one non-rodent species) unless there is one species generally accepted as the best model for humans. The end points used in the animal efficacy studies must be clearly related to desired benefit in humans (e.g. mortality or serious morbidity).

The development and validation of animal models of human radiation injury present some serious challenges. The process may be particularly difficult if the potential therapy is intended to protect, mitigate or treat a specific organ system for which there are currently no approved therapies for use as ''gold standards.'' Animal model development and interpretation may be further confounded by interspecies differences, and if validation requires nonhuman primates, their relative scarcity could further delay development. As promising agents emerge, their effect on carcinogenesis must be assessed, and those that have potential for use in radiation oncology must also be evaluated for their effect on tumor radioresponse.

ONGOING ACTIVITIES

A request for applications (RFA) was issued in October 2004 by the National Institute of Allergy and Infectious Diseases (NIAID) for Centers for Medical Countermeasures Against Radiation (see NIH Guide for Grants and Contracts, Notice NOT-AI-04-027, April 9, 2004, http://grants1.nih.gov/grants/guide/notice-files/NOT-AI-04-027.html), with applications to be received in February 2005 and awards made by September 2005.

An additional workshop on ''Animal Models for Radiation Injury, Protection and Therapy'' was held May 25–26, 2004 in Bethesda, MD and was sponsored by NIAID.

Discussions are under way with the FDA regarding preclinical end points, such as lethality endpoints.

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